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ABSTRACT:

Cyanogenesis in 50 species of vascular plants collected from east central Illinois was studied by examining 10 populations of each species. Each population consisted of 30 individuals, which gave an over all sample size of 300 plants per species. Herbarium specimens from the Stover Herbarium of Eastern Illinois University (EIU) were also tested for the presence of cyanide. More than 20,000 specimens were tested for this study which included cultivated plants, introduced weeds, and native plant species. The speed and degree of the HCN reaction as well as the specific plant part tested were included to give a more specific account of cyanogenesis.

Some species examined had a very high cyanogenic frequency with most populations reaching 100% cyanogenesis. Included in this group are Coronilla varia, Cystopteris fragilis, Gillenia stipulata, Hordeum jubatum, Isopyrum biternatum, and Prunus serotina. Several other species had very low cyanogenic frequencies, such as Achillea millefolium, Avena sativa, Fragaria virginiana, Glycine max, Potentilla recta, Rosa multiflora, and Tilia americana. Arisaema triphillum, Delphinium tricorne, and Sambucus canadensis are examples of highly variable cyanogenic species. Included in this study are several important cultivated crop and forage plants. Representatives of this group are Avena sativa, Glycine max, Medicago sativum, Trifolium hybridum, T. pratense, T. repens, Triticum aestivum, and Zea mays.

In the present study 24 previously unreported species were found to be cyanogenic. Also, 26 previously reported species were examined to determine their cyanogenic frequencies. It may be concluded from this study that the frequencies of cyanogenesis are usually

inconsistent within any particular species. Also, the hydrogen cyanide content is highest in young shoots and leaves, and there is a higher frequency of HCN in the native Illinois species than in cultivated and introduced plants.

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INTRODUCTION:

Many plants have the ability to synthesize compounds which are capable of liberating hydrogen cyanide gas upon hydrolysis of plant tissue. This phenomenon, known as cyanogenesis, has been recognized for more than a century and is not restricted to a particular group of plants. Cyanogenesis has been reported in bacteria, fungi, ferns, gymnosperms, as well as monocotyledonous and dicotyledonous angiosperms. Among higher plants these compounds are generally restricted to the leaves but also occur in roots, stems, seeds, flowers, and other plant parts. Most cases of cyanide poisoning are caused by plants in the Rosaceae, Leguminoseae, Euphorbiaceae (primarily cassava), or members of the genus Sorghum (Poaceae). Presently cyanide has been discovered in at least 2,050 species of plants representing about 110 families (Seigler, 1981).

Cyanogenesis is caused by a cyanoglycoside or cyanolipid, which are compounds that yield one or more sugars or fatty acids and one or more other compounds or aglycones upon hydrolysis. Hydrogen cyanide is an aglycone which is only liberated in the presence of a certain specific enzyme, and the amount of cyanide will depend on several intrinsic (genetics, part of plant, age of plant, and sometimes the sex of the plant) and extrinsic factors (climate, moisture supply, soil fertility, and freeze damage) (Kingsbury, 1964). More than 30 different cyanogenic compounds have been reported, but specific compounds have been isolated from less than 100 species (Seigler, 1977). The present study was undertaken to determine the frequency of cyanogenesis within populations of a broad range of vascular plant species. The percentage of cyanogenesis within these populations will

give some indication of polymorphism within a species, and be useful in understanding these species from a chemotaxonomic viewpoint. There are few previous studies which have dealt directly with frequencies of cyanogenesis at the population level. Populations of Trifolium repens, Lotus corniculatus, and Lotus alpinus have been studied in detail (Daday 1954a, 1954b, 1965; Jones 1970, 1972, 1973; Foulds and Grime 1972a, 1972b; Araujo 1976; Urbanska and Wildi 1975; Urbanska et al. 1979; Urbanska and Schwank 1980). Other detailed population studies were done with Lotus alpinus and Ranunculus montanus which dealt with polymorphism of cyanogenesis on different soil substrates, and at various elevation levels (Urbanska, 1982; and Dickenmann, 1982).

MATERIALS AND METHODS:

More than 20,000 specimens, both fresh and dried, collected from east central Illinois were tested for the presence of cyanogenic compounds. Thirty specimens from a single population and a total of ten populations for each species were collected to give a representative sample size of 300 for each species. Also, all herbarium specimens of each species in the Stover Herbarium of Eastern Illinois University (EIU) were tested for the presence of cyanide. Each of these specimens were tested for the presence of cyanogenic compounds by the method previously described by Feigl and Anger (1966) and modified by Tantisewie et al. (1969). In order to conduct this test, a small amount of plant material (about 200 mg) is crushed, placed in a vial, and moistened with distilled water. A strip of filter paper impregnated with copper ethylaceoacetate and tetra base (4,4'-tetramethyldiaminodiphenylmethane) is added to the vial so as not

to touch the sample, and the vial sealed with a cork. (Caution must be taken when reusing these corks. They must be washed thoroughly and allowed to sit for 4 or 5 days to eliminate any possibility of false positive results.) The presence of cyanide is indicated if the filter paper turns a deep blue color within a 24 hour period. A negative test indicates the specimen tested does not contain a cyanogenic compound or lacks the enzyme capable of hydrolyzing the cyanogenic compound, or both. Nomenclature follows Mohlenbrock (1975).

RESULTS AND DISCUSSION:

Among the 50 plant species tested variation exists in the frequency of cyanogenesis, the speed at which cyanide is released, the amount of cyanide produced, and the parts of the plant that are cyanogenic (Table 1). In most species young leaves and shoots gave the strongest reaction, but mature leaves, as well as other plant parts can also be cyanogenic. In some of the species, such as Platanus occidentalis the young developing leaves are usually strongly cyanogenic while mature leaves usually test negative for HCN production. Similar results were obtained for Celtis occidentalis, Tilia americana, Heracleum maximum, and Lolium perenne. In other species, particularly Hordeum jubatum, Isopyrum biternatum, Coronilla varia, Cystopteris fragilis, and Prunus serotina both young and mature leaves were equally cyanogenic. In Arisaema triphyllum leaves of many individuals were tested, all with negative results, while the spadix and flowers proved to be occasionally cyanogenic. In other species young stems and very small leaves were tested with positive results. Included in this group are Veronica arvensis, Gillenia stipulata,

Achillea millefolium, Coronilla varia, Chenopodium album, Medicago sativa and Vicia villosa. Seigler (1987), Gibbs (1974), and Tjon Sie Fat (1979) reported that the age of the material and the plant part tested can determine the degree of cyanogenesis. In the present study the best results were obtained by testing young shoots and young leaves.

Cyanogenesis can be strongly affected by environmental factors, plant age, growth phases of the plant and the plant part used for the test. Environmental conditions are able to suppress or to induce cyanogenesis in plants which have a cyanophoric potential and local selection pressure. It has also been shown that many grasses, which are perfectly good fodder plants under normal conditions, become toxic due to HCN being release when the plant is wilting (Henrici, 1926). Also, drought stress and rapid regrowth after long periods of drought may have a significant effect on cyanogenesis in many grasses, and the intensity of cyanogenesis due to environmental stresses may be under genetic control (Steyn, 1934; van der Walt & Steyn, 1941). In Juncus (Zandee, 1976) cyanogenesis is strongly affected by plant age, plant parts, developmental stages, and to a lesser extent ecological influences (Zandee, 1983).

A quantitative determination of cyanide was not made in the present study. An indication of the amount of cyanide produced, however, can be made by observing the extent of the color change of the Feigl/Anger paper (Dickenmann, 1982). In this study the reaction was considered weak if only part of the paper turned a light blue, moderate if it turned a light to medium blue, and strong if the paper turned a deep blue. According to Dickenmann (1982), the weak reaction contains

approximately 2-20 mg of HCN per Kg fresh weight, while the moderate reaction produces 21-50 mg of HCN per Kg fresh weight, and the strong reaction produced more than 50 mg of HCN per Kg fresh weight. Seven of the species tested consistently gave a strong reaction for cyanide. This grouping includes: Cystopteris fragilis , Gillenia stipulata, Hordeum jubatum, Isopyrum biternatum, Platanus occidentalis, Prunus serotina, and Trifolium repens. The majority of the species tested are weak (15 species) or moderately (12 species) cyanogenic. Some species, however, are highly variable (16 species) in the amount of HCN produced. In these species, some of the individuals in a population were only weakly cyanogenic, while others in this same population gave moderate or even strong reactions. Some of the species in this category are Avena sativa, Barbarea vulgaris, Cardamine pensylvanica, Poa pratensis, Sambucus canadensis, Trifolium hybridum, and Triticum aestivum (Table 1). The speed of the reaction was also highly variable among the species examined. Several species gave a fast, very strong reaction within 30 minutes. Included in this group are Cystopteris fragilis, Gillenia stipulata, Isopyrum biternatum, Platanus occidentalis, Prunus serotina, and Trifolium repens. The remaining species had a moderate to slow reaction time with variable reaction strengths. One species tested, gave a fast but weak reaction. Coronilla varia was 100% cyanogenic in 80% of the populations studied with mostly a weak, fast cyanide reaction. On the Feigl/Anger paper this weak, light blue, reaction was then replaced by a pale orange color. This pale orange color reaction following the cyanide reaction was also observed in Glycine max, and most members of the Cruciferae examined. In the Cruciferae species the cyanide reaction was usually

pushed upwards on the Feigl/Anger paper leaving behind either no color at all, or a pale orange to brick red color. Another unusual reaction was a black color developing after the fast cyanide reaction in Platanus occidentalis.

In some cases if the test was extended 48 hours it was not uncommon for more of the individual species to test positive and other positives to become stronger. A good example of this was in Asclepias amplexicaulis which was mostly weak at 24 hours, and would give a very strong reaction at the end of 48 hours. The tests for this study were limited to 24 hours to eliminate false positive results due mainly to bacterial and fungal contamination.

For most of the species examined the frequency of cyanogenesis proved to be extremely low in nearly all populations (Table 1). Twenty nine of the species examined had no populations with a frequency over 50 percent. In the case of Potentilla recta none of the populations studied proved to be cyanogenic, while 2 herbarium specimens produced a positive but weak reaction after 24 hours. Other species like Fragaria virginiana had a very low cyanogenic frequency with only three populations having a 3 percent frequency and the remaining populations lacking HCN altogether. Festuca pratensis was much the same but with a high percentage (7%), and 50% of the populations having no HCN positive individuals. Other species examined had no populations with a cyanogenic frequency over 10% (Achillea millefolium, Anemone canadensis, Ranunculus septentrionalis, and Rosa multiflora). Conversely, there were several species that had population frequencies greater than 50%, with some as high as 100%, and other populations lacking HCN altogether. Some examples of this group are Arisaema

triphyllum, Barbarea vulgaris, Delphinium tricorne, Myosurus minimus, Poa pratensis, Salix interior, Sambucus canadensis, and Zea mays.

Included in this study were several economically important cultivated crop plants. It should be noted that only leaf material; no seeds or fruits were tested. Avena sativum had a cyanogenic frequency of 17% in one population while 70% of the populations studied were not cyanogenic. Also, following this pattern of low HCN frequency was Glycine max. It had a frequency of 13% in one population, but 40% of the populations were negative for cyanogenesis. In contrast, Triticum aestivum had cyanogenic individuals in all 10 populations, and some populations had a frequency as high as 43%. The most variation was seen in Zea mays. One population scored was 60% cyanogenic, while half of the populations studied did not produce cyanide.

Another economically important group of plants, the forage and lawn plants, were also tested. Included in this group are Coronilla varia, Festuca pratensis, Lolium perenne, Medicago sativum, Poa pratensis, Trifolium hybridum, T. pratense, and T. repens. With the exception of Coronilla varia and Trifolium repens, these eight species are introduced weeds and cover crops with low cyanogenic frequencies. Six species in this group proved to be consistently low in cyanide production with cyanogenic frequencies averaging below 20%.

Herbarium specimens of each species examined were also tested (Table 1). This information proves to be valuable for the testing of new species and obtaining habitat, locational, and seasonal data. Since the cyanogenic compounds can remain intact for several decades, the testing of herbarium specimens is very easy and accurate. The column marked Herbarium (table 1) gives the number of positive HCN

tests, and separated by a slash is a second number which represents the total number of specimens tested. In most cases the percent cyanogenic individuals was lower in the herbarium study, but it gives a good indication of cyanogenic species.

The majority of the species tested (31) are native Illinois plants, while 4 are cultivated species and 15 are introduced weeds or forage plants. The cultivated species tested have overall a relatively low frequency of cyanogenesis as compared to the high HCN concentrations of the native Illinois plants. Although there are still variations in the native Illinois group, the native plants still maintain the greatest ability to produce cyanogenic compounds. Of the weedy species tested there were some highly cyanogenic populations, but they also possessed a high degree of variability. This group includes Barbarea vulgaris, Cardamine pensylvanica, Chenopodium album, Delphinium tricorne, Myosurus minimus, Poa pratensis, Salix interior, and Sambucus canadensis.

Table 1: Listing of the 50 cyanogenic species tested, first reported reference, speed of cyanogenic reaction, degree of cyanogenic reaction, plant part used in testing, and also included are the population data of 10 separate populations and herbarium specimens tested.

SPECIES	REFERENCE	Speed of Reaction*	Degree of Reaction**	Part Tested***	Percent Cyanogenic in Population #										Herbarium****
					1	2	3	4	5	6	7	8	9	10	
Achillea millefolium L.	14, 15	S	W	L/S	10	7	0	0	0	0	0	0	0	0	0/36
Anemone canadensis L.	11	M	W	L	3	0	0	0	0	0					0/17
Arisaema triphyllum (L.) Schott	This Study	M	V	SP	90	33	27	23	23	3					0
Asclepias amplexicaulis Sm.	This Study	S	W	L	47	33	23	19	6	0	0	0			0/06
Avena sativa L.	22	S	V	L/S	17	13	7	0	0	0	0	0	0	0	1/15
Barbarea vulgaris R. Br.	This Study	M	V	L	100	63	50	43	13	7	7	3	3	3	1/55
Caltha palustris L.	This Study	S	V	L/S	33	27	23	23	20	13	7				4/20
Cardamine pensylvanica Muhl.	This Study	M	V	L	100	100	83	53	50	48	20	13	7	0	8/33
Celtis occidentalis L.	This Study	S	V	L	40	21	13	8	4	0	0	0	0	0	0/51
Chenopodiun album L.	21	M	V	L/S	60	37	20	17	13	7	3	0	0	0	0/24
Coronilla varia L.	This Study	F	W	L	100	100	100	100	100	100	100	100	50	7	0/12
Cystopteris fragilis (L.) Bernh.	13	F	S	F	100	100	100	100	100	97	97	90	80	47	37/52
Delphinium tricorne Michx.	This Study	M	V	L	97	57	33	33	10	4	3	0	0	0	18/40
Festuca pratensis Huds.	7	S	W	L	7	3	3	3	3	0	0	0	0	0	0/37
Fragaria virginiana Duch.	This Study	S	W	L	3	3	3	0	0	0	0	0	0	0	3/45
Gillenia stipulata (Muhl.) Baill	This Study	F	S	L	100	100	100	100	100	93	90	80	77	20	11/31
Glycine max (L.) Merr.	11	S	M	L	13	10	3	3	3	3	0	0	0	0	0/08
Hepatica nobilis Schreb.	This Study	S	W	L	23	10	7	3	0	0	0	0	0	0	1/31
Heracleum maximum Bartr.	This Study	M	M	L	43	40	33	13	7	3	0	0	0		0/11
Hordeum jubatum L.	22	M	S	L	100	100	100	100	100	100	100	100	100	57	11/46

Table 1 (Continued)

Isopyrum biternatum (Raf.) T. & G.	16	F	S	L	100	100	100	100	100	100	100	100	100	100	27/38
Lolium perenne L.	22	S	W	L	27	10	3	3	3	0	0	0	0		0/14
Medicago sativa L.	16	S	M	L	27	17	17	13	13	10	7	3	0	0	6/36
Menispermum canadense L.	11	S	W	L	37	10	10	7	0	0	0	0	0	0	0/56
Mertensia virginica (L.) Pers.	This Study	S	M	L	30	13	10	5	0	0	0				0/28
Myosurus minimus L.	11	S	M	L	80	60	27	23	23	13	8	3	3	0	6/30
Oenothera biennis L.	27	M	W	L	20	3	0	0	0	0	0	0	0	0	0/57
Phlox divaricata L.	This Study	S	M	L	30	23	23	20	17	13	10	10	7	7	2/62
Platanus occidentalis L.	12	F	S	L	100	100	100	89	89	54	35				0/36
Poa pratensis L.	26	S	V	L	70	10	3	0	0	0	0	0			1/39
Polystichum acrostichoides Schott.	This Study	S	M	F	20	13	10	10	3	3	0	0	0	0	0/64
Potentilla recta L.	This Study	S	W	L	0	0	0	0	0	0	0	0	0	0	2/43
Prunus serotina Ehrh.	26	F	S	L	100	100	100	100	100	100	100	100	100	100	53/67
Ranunculus abortivus L.	This Study	S	W	L	23	10	7	5	3	3	0	0	0	0	11/66
Ranunculus septentrionalis Poir.	This Study	S	W	L	10	10	7	0	0	0	0	0	0	0	5/45
Rosa multiflora Thunb.	This Study	S	W	L	10	7	7	7	3	0	0	0	0	0	2/29
Salix interior Rowlee	2	V	V	L	100	57	13	12	10	3	0	0	0	0	9/46
Sambucus canadensis L.	This Study	M	V	L	90	13	12	10	3	0	0	0	0	0	4/53
Thalictrum dioicum L.	29	S	M	L	40	20	17	3	0	0	0	0	0	0	0/25
Thalictrum revolutum DC.	This Study	F	M	L	92	48	47	2							2/22
Thlaspi arvense L.	16	S	M	L	87	40	37	13	3	3	0	0	0	0	0/26
Tilia americana L.	This Study	S	V	L	13	13	0	0	0	0					0/46
Trifolium hybridum L.	25	M	V	L	40	37	33	13	10	3	0	0	0	0	6/32
Trifolium pratense L.	7	S	M	L	23	20	17	13	10	7	3	0	0	0	2/43
Trifolium repens L.	23	F	S	L	97	97	80	53	40	37	33	27	23	23	20/24
Triticum aestivum L.	22	S	V	L	43	40	40	40	37	27	13	10	10	7	0/16
Uvularia grandiflora Sm.	This Study	S	W	L	17	10	5	3	3	0	0	0	0	0	0/50
Veronica arvensis L.	This Study	S	V	S	30	20	17	13	10	3	3	3	0	0	0/43
Vicia villosa Roth.	26	S	V	L	17	10	3	3	0	0	0	0	0	0	4/20
Zea mays L.	26	S	M	L	60	17	13	7	0	0	0	0	0	0	0/01

*S = Slow, M = Medium, F = Fast, V = Variable
**W = Weak, M = Moderate, S = Strong, V = Variable
*** S = Shoots, F = Frond, L = Leaves, SP = Spadix
****(# positive/total # tested)

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